

# ***Office of Biological and Environmental Research***

## ***Biological Systems Science Division Update***

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Director, Biological Systems Science Division,  
Department of Energy, Office of Biological &  
Environmental Research

March 22, 2016



U.S. DEPARTMENT OF  
**ENERGY**

Office  
of Science

Office of Biological  
and Environmental Research

# *Programmatic Activities*

## ***Completed Reviews/Activities***

- Annual reviews of the three Bioenergy Research Centers (BRCs)
- Early Career panel reviews - (Feb 4)
- Annual Genomic Sciences Program PI Meeting – (Mar 7-9)

## ***Upcoming Reviews/Activities***

- Panel Review of Plant Feedstocks for Bioenergy FOA (April)
- Panel review for Carbon Cycling FOA (May)
- ORNL Bioimaging project review (Jun 2)
- Three National Laboratory Science Focus Area (SFA) reviews (Aug- Sept)
- Workshop (TBD Sept)

## ***New FOAs for FY 2016***

### ***Plant Feedstock Genomics for Bioenergy: A Joint Research Funding Opportunity Announcement USDA, DOE (DE-FOA-0001444)***

- *Continues research on plant responses to pathogens*
- *Includes a focus on oil seed crops*

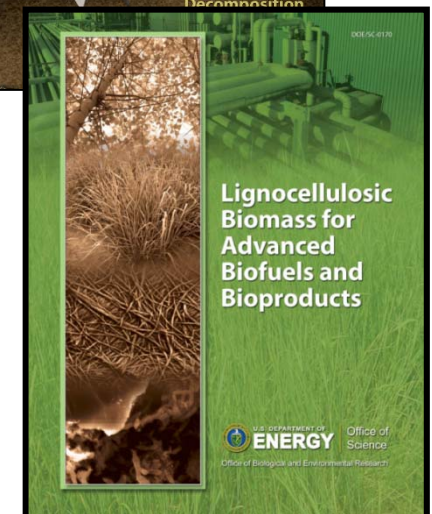
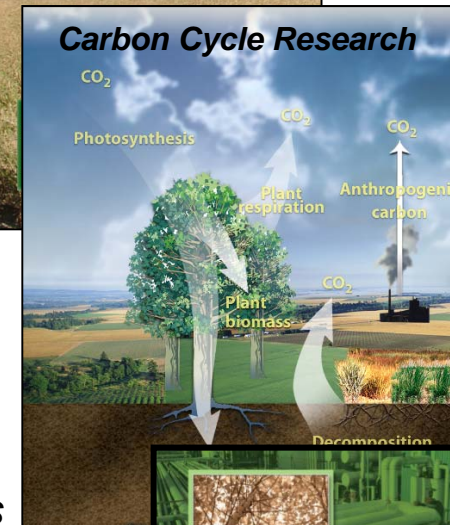
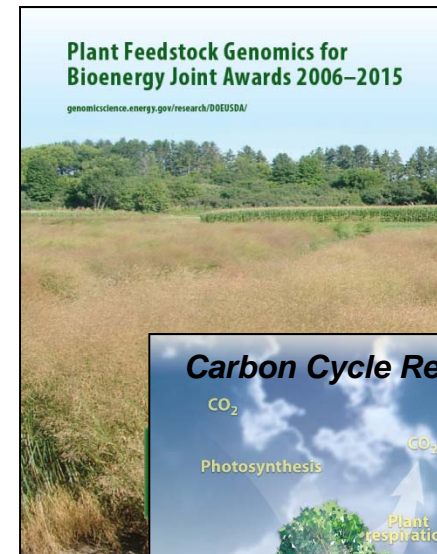
### ***Systems Biology Enabled Research on the Roles of Microbial Communities in Carbon Cycle Processes (DE-FOA-0001458)***

- *Research to understand the functioning of microbial communities in a wide range of environments and environmental conditions*
- *Research to inform larger scale ecosystem function*

### ***Later this Fiscal Year***

### ***Bioenergy Research Centers FOA (open competition)***

- *Informed by a recent BER workshop on bioenergy research*



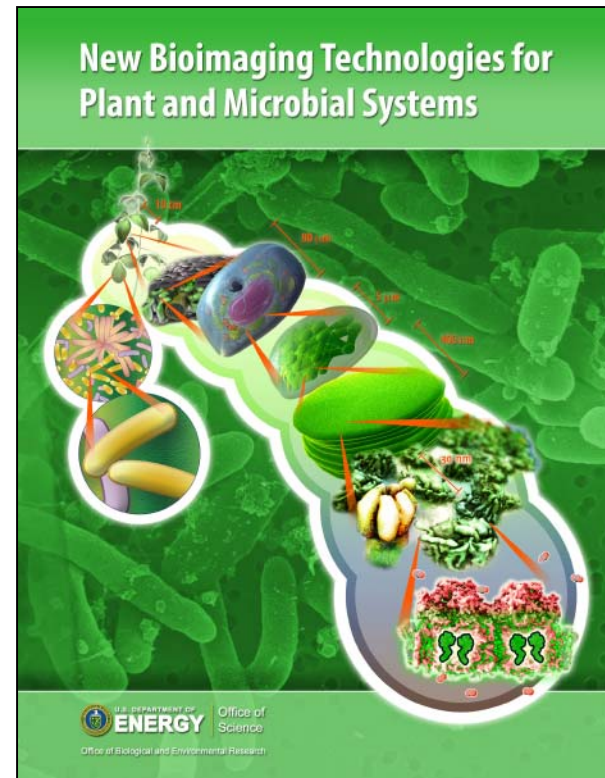
# Bioimaging Technology Program

## DOE National Laboratories\*

- **Argonne National Laboratory**  
Kenneth Kemner, Mark Hereld
- **Oak Ridge National Laboratory**  
Mitchel Doktycz
- **Pacific Northwest National Laboratory**  
James Evans
- **SLAC National Accelerator Laboratory**  
Soichi Wakatsuki

## Universities

- **Duke University**  
Tuan Vo-Dinh
- **Iowa State University**  
Basil Nikolau
- **Texas A&M University**  
Wayne Versaw
- **University of Connecticut**  
Leslie Shor
- **University of Missouri, Columbia**  
Gary Stacey
- **University of North Carolina at Chapel Hill**  
Elizabeth Shank
- **Washington University in St. Louis**  
David Fike



[http://science.energy.gov/~media/ber/pdf/community-resources/bioimaging\\_technologies.pdf](http://science.energy.gov/~media/ber/pdf/community-resources/bioimaging_technologies.pdf)

***\*Opportunity for an additional project in FY16***

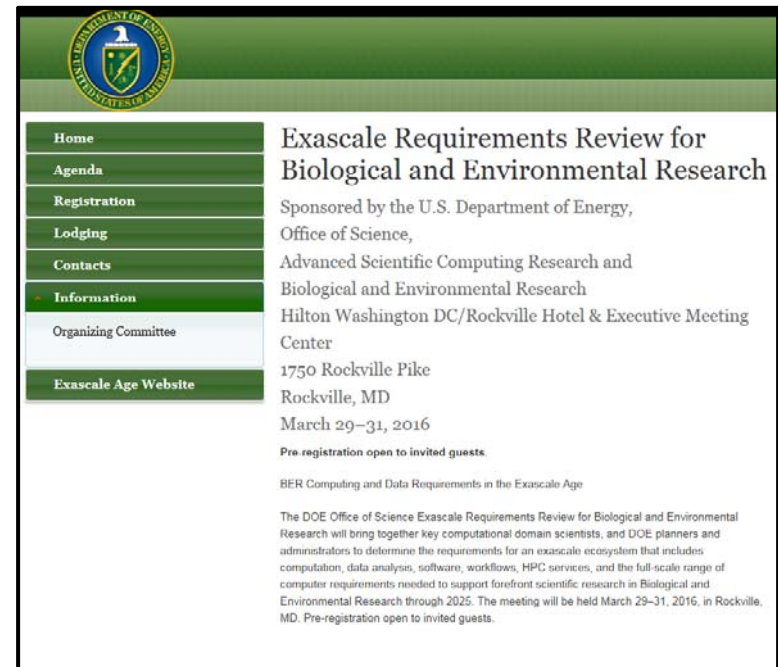
# Strategic Planning: New Workshops

## ASCR-BER Workshop on Exascale Computational Requirements

- March 29-31, 2016
- Hilton Washington DC/Rockville Hotel

## Workshop on Integrated Molecular Structure, Function and Dynamic Imaging Technologies

- Enabling *in situ* Quantitative Biology
- Date – mid-September, 2016
- Venue – TBD
- Workshop approval submitted





# The DOE Systems Biology Knowledgebase

## A Community Resource for Predictive Biology



Open software and data platform for addressing the grand challenge of systems biology: **Predicting and designing biological function**

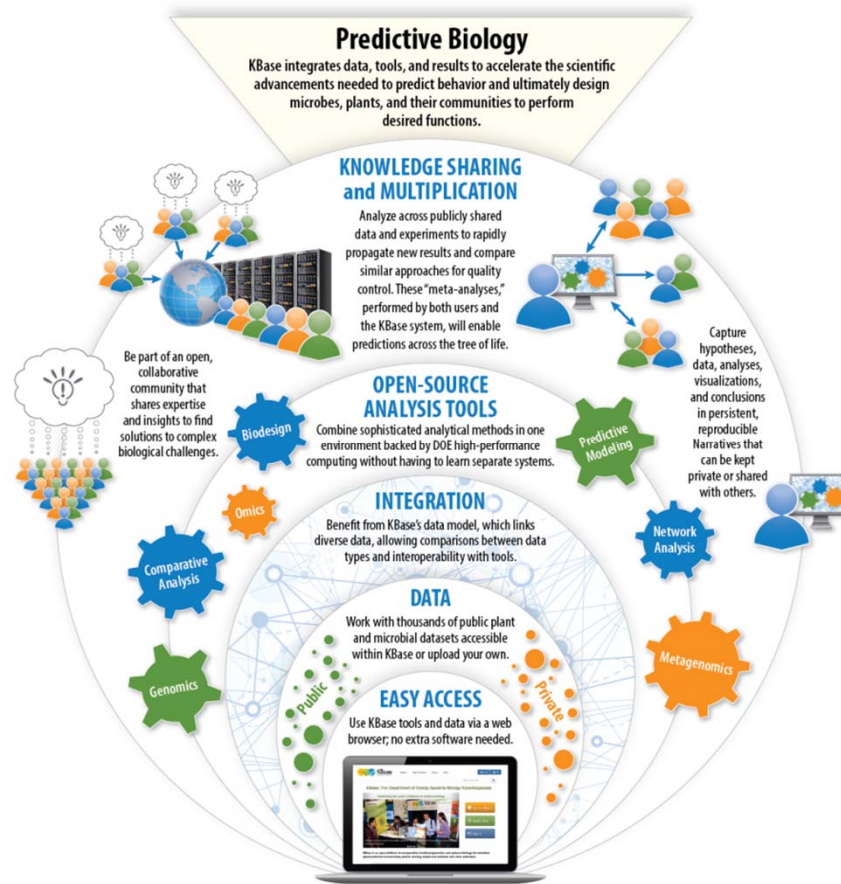


Unified system that integrates data and analytical tools for comparative functional genomics of **microbes, plants, and their communities**



Collaborative environment for **sharing methods and results** and placing those results in the context of knowledge in the field

**New Feature!** KBase Software Development Kit (SDK) ver.1 for Third Party Tool Integration



DOE Systems Biology Knowledgebase

<http://www.kbase.us>

**Demonstrations of the latest version at the 2016 Genomic Sciences Annual PI Meeting March 7-9**

# Bioenergy Research Centers (BRCs)

## Overall Scientific goals for FY 2016

### Great Lake Bioenergy Research Center (GLBRC)

- Evaluate transgenics for biosynthesis genes, transcription factors and, promoters for increased hemicelluloses in stem parenchyma tissues
- Design optimal 'drop-in' fuel plant oils and direct production to vegetative tissues
- Evaluate optimal enzyme mixtures for recalcitrant species

### Joint BioEnergy Institute (JBEI)

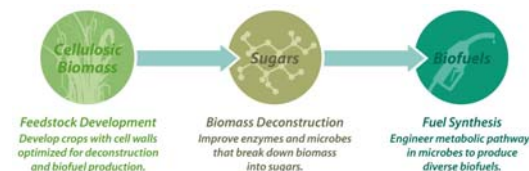
- Optimize pathways for new lignin traits using synthetic biology tools from Fuels Synthesis Division
- Demonstrate conversion of lignin-derived aromatics to methyl ketones
- CRISPR-based chromosomal integration and systematic optimization of isoprenoid pathway in *E. coli*

### BioEnergy Science Center (BESC)

- Incorporate omics data into genome-scale metabolic network to identify reaction bottlenecks and improve strain design of *C. thermocellum* for enhanced biofuels production
- Identify correlations between the chemical properties and altered recalcitrance in TOP Lines using advanced characterization methods
- Improve the *C. thermocellum* cellosome and determine the synergy of *C. thermocellum* cellosomes with heterologous cellulolytic enzymes

#### **To date the BRCs have generated :**

- 825 invention disclosures and/or patent applications
- 42 patents awarded
- 129 licensing agreements
- 2187 peer-reviewed publications



## Optimizing Biocatalyst-Feedstock Combinations to Achieve High Solubilization with Minimal Pretreatment

### Objective

Demonstrate lignocellulosic solubilization by various biocatalysts

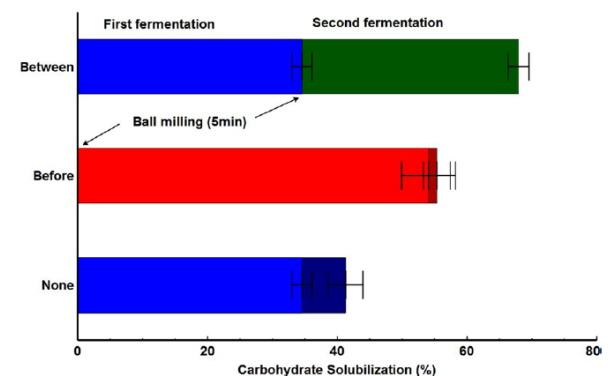
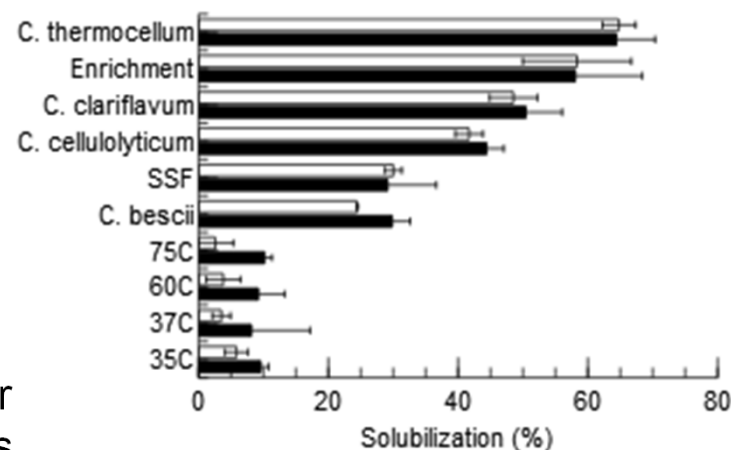
### Approach

Trends drawn from time-course and end-point data for six conversion systems and three substrates were:

- Greater-than-expected differences between biocatalysts
- Lower yields and greater dependence on particle size for *Populus* as compared to green or senescent switchgrass
- Equal fractional solubilization of glucan and xylan
- Two-fold increase in solubilization via mechanical disruption with greater benefits on partially-fermented feedstock.

### Results/Impact

- Some biocatalysts are more effective at achieving high solubilization with minimal pretreatment.
- Starting with nature's best biomass-solubilizing systems may enable a reduction in the amount of non-biological processing required, and in particular substitution of cotreatment for pretreatment.



Paye, Julie M.D., et al., "Biological lignocellulose solubilization: comparative evaluation of biocatalysts and enhancement via cotreatment," *Biotechnol Biofuels*, 9:8, 2016. DOI 10.1186/s13068-015-0412-y





## *A Novel Lipid Pathway is Responsible for the Massive Quantity of Surface Wax on Bayberry Fruit*

### **Objective**

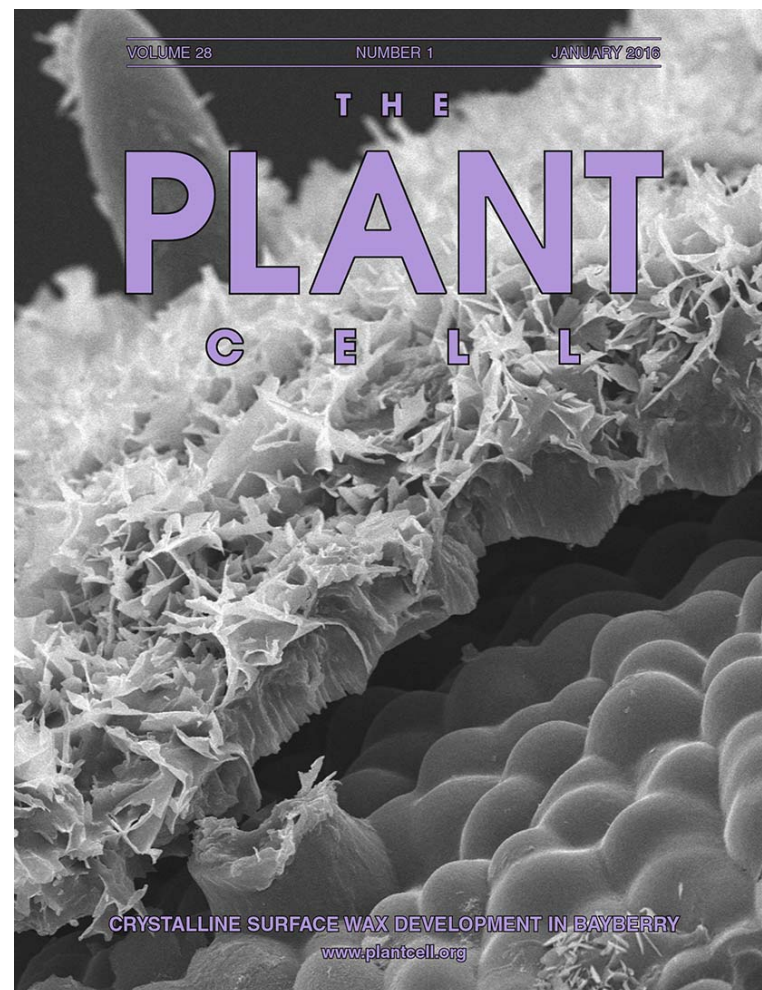
To discover how Bayberry achieves the highest level of surface lipid known in nature

### **Approach**

- Analyze morphological and chemical development of surface wax
- Monitor wax biosynthesis with [ $^{14}\text{C}$ ]-radiolabeling to determine mechanisms of synthesis
- Use RNA-seq to identify highly expressed transcripts that may contribute to surface wax production
- Propose a model for Bayberry wax biosynthesis

### **Result/Impacts**

- Extracellular glycerolipids in Bayberry wax are made by a novel triacylglycerol synthesis pathway
- An increased understanding of this process may be useful in engineering plants for accumulation and secretion of high value lipids and other hydrocarbons



Simpson JP, Ohlrogge JB (2016) A Novel Pathway for Triacylglycerol Biosynthesis Is Responsible for the Accumulation of Massive Quantities of Glycerolipids in the Surface Wax of Bayberry (*Myrica pensylvanica*) Fruit. *The Plant Cell* 28, 248-264.

# Evolved Hexose Transporter Enhances Xylose Uptake and Glucose/Xylose Co-utilization in *Saccharomyces cerevisiae*

## Objective

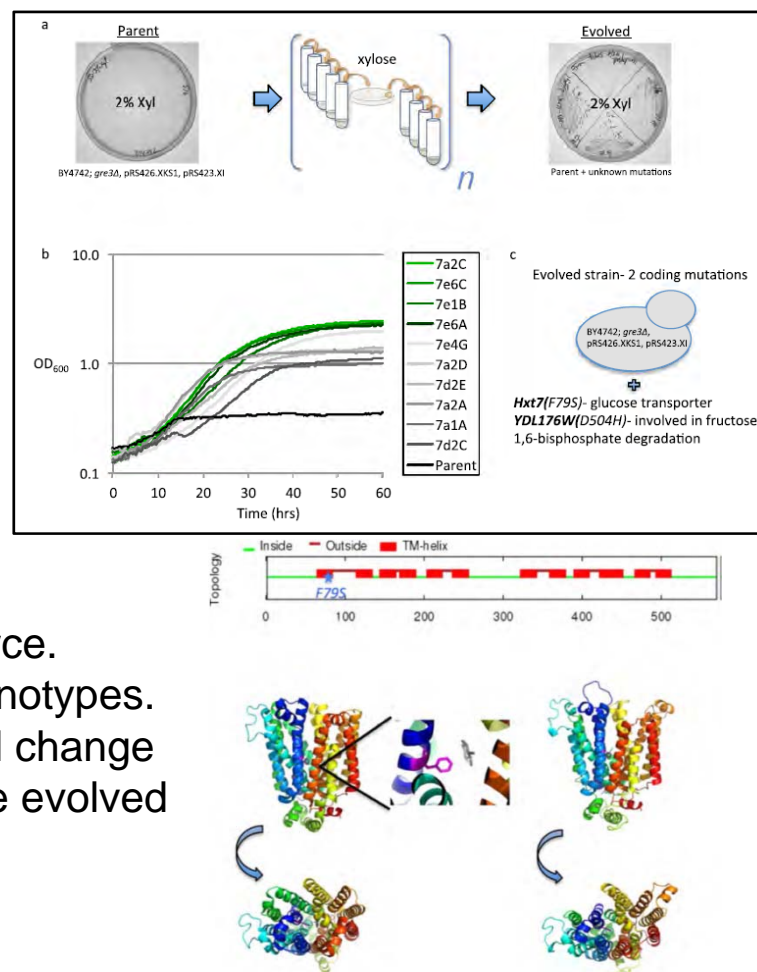
Develop methods to broaden the use of sugars in the typical biomass mixture by *Saccharomyces cerevisiae*

## Approach

- Import of five carbon sugars into the cell remains an important bottleneck
- *S. cerevisiae* strain BY4742 strain is engineered with a heterologous xylose utilization pathway
- Constructed strain is then subjected to a laboratory evolution regime with xylose as the sole carbon source.
- Several strains recovered with improved growth phenotypes.
- Genome resequencing identified a single amino acid change in the hexose transporter (*HXT7*) responsible for the evolved phenotype.

## Results/Impact

- Constructed mutant (*HXT7*(F79S)) shows improved xylose uptake rates that allows the *S. cerevisiae* strain to show significant growth with xylose as the sole carbon source, as well as partial co-utilization of glucose and xylose in a mixed sugar cultivation.



Apel, Amanda Reider et. al., "Evolved hexose transporter enhances xylose uptake and glucose/xylose co-utilization in *Saccharomyces cerevisiae*." **Nature Science Reports** 6:19512 (2016) [DOI: 10.1038/srep19512]

## ***Using Meta-Omics Techniques to Track Shifting Microbial Community Processes in Warming Permafrost***

### **Objective**

Examine how experimental warming alters the structure and functional properties of microbial communities in permafrost.

### **Approach**

Use metagenomic/transcriptomic analysis to track changes in functional microbial community structure and correlate with shifts in environmental processes during ~0.6 - 2.3°C warming over 1.5 years.



### **Results/Impact**

- Warming resulted in shifts in the overlying plant community, changes in soil moisture and carbon content, and significant increases in gross primary productivity (+30%) and ecosystem respiration (+38%) that resulted in a net carbon loss.
- Species level microbial community composition did not significantly change, but expression of functional genes involved in decomposition of soil carbon (labile and recalcitrant), methanogenesis, and denitrification increased in warmed plots.
- These findings demonstrate the importance of investigating shifts in microbial functional gene expression as well as community composition to understand climate change impacts.

## Deciphering Senescence in Switchgrass

### Objective

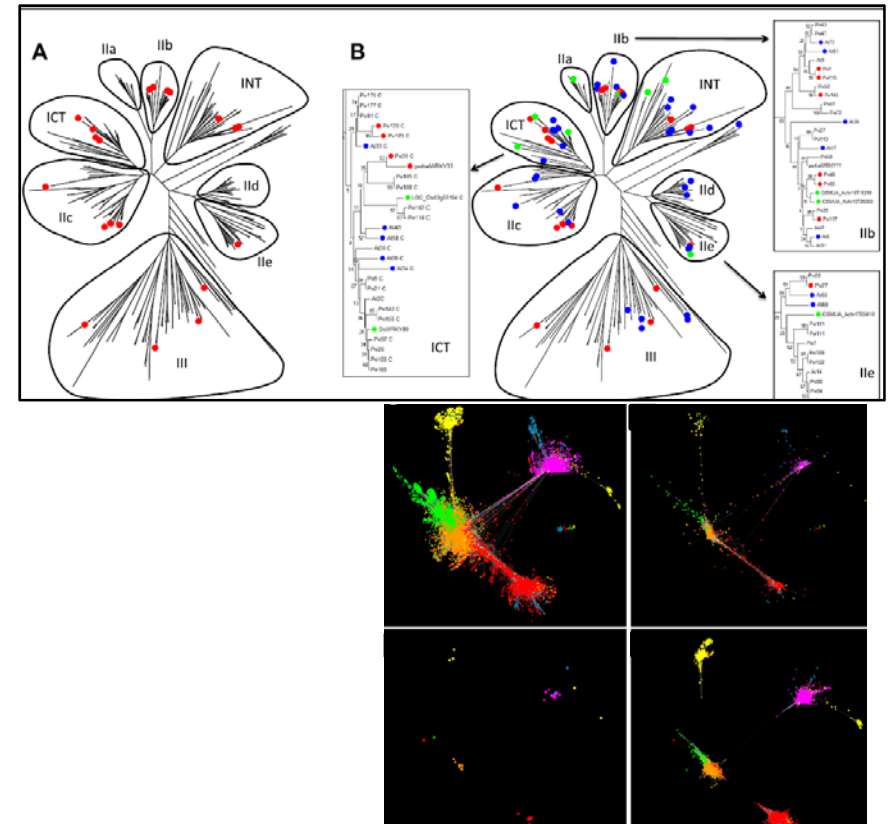
Determine the genetic markers for senescence in perennial switchgrass (*Panicum virgatum*).

### Approach

- Researchers mined the switchgrass genome to identify regulatory genes called WRKY transcription factors.
- WRKY genes are known to be involved in several plant processes, including induction of senescence.
- Using transcriptome analysis the researchers identified 23 specific WRKY genes whose expression was elevated in the senescence-associated modules, eleven of which were found in “hotspots” of related senescence-associated genes from multiple species.

### Results/Impact

- These genes can be targeted for functional characterization and for marker development, providing a foundation for genetic improvement of switchgrass as a bioenergy crop.



C. I. Rinerson, et al., "The WRKY transcription factor family and senescence in switchgrass".  
**BMC Genomics**. 16:912 (2015). DOI: 10.1186/s 12864-015-2057-4



## *Engineered yeast gene drives that can be safely contained and removed*

### **Objective**

Engineer a gene drive that functions in yeast but can be readily biocontained or reverted.

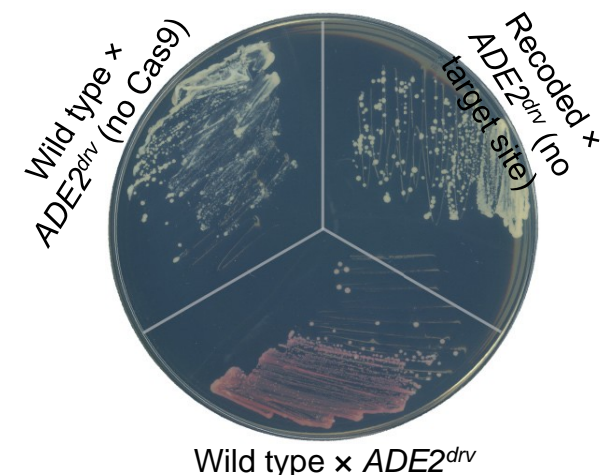
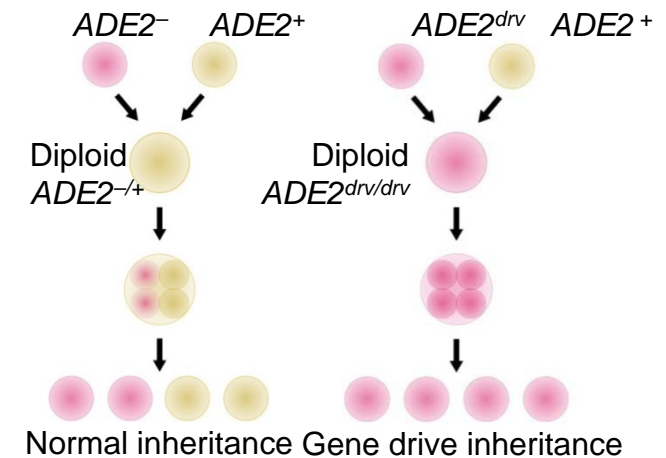
### **Approach**

A CRISPR-Cas9 based gene drive was designed to target the yeast *ADE2* gene (pink). In order to prevent this gene drive from escaping into the environment and affecting wild yeast populations, only the *ADE2*-targeted guide RNA sequence is integrated into the host genome while the Cas9 nuclease is coded in a selectable plasmid. In this way, a 99% inheritance of the gene drive was obtained while wild type *ADE2* was normally inherited in the absence of the Cas9 plasmid. Furthermore, another gene drive was designed to restore the natural version of the *ADE2* targeted gene and to remove any remaining gene drive DNA from the genome.

### **Results/Impact**

- Gene drives can be designed for sexually reproduced unicellular organisms.
- Molecular bioconfinement approaches are effective to control gene drives.
- These results are a step forward toward a safe use of engineered gene drives to advance DOE missions.

DiCarlo, Chavez, Dietz, Esvelt, Church. Safeguarding CRISPR-Cas9 gene drives in yeast. **Nature Biotechnology** 33: 1250 (2015).







**PECase Winner!**  
**Michelle O'Malley (UCSB)**

## *New, highly effective biomass-degrading enzymes discovered in anaerobic fungi from herbivores' guts*

### Objective

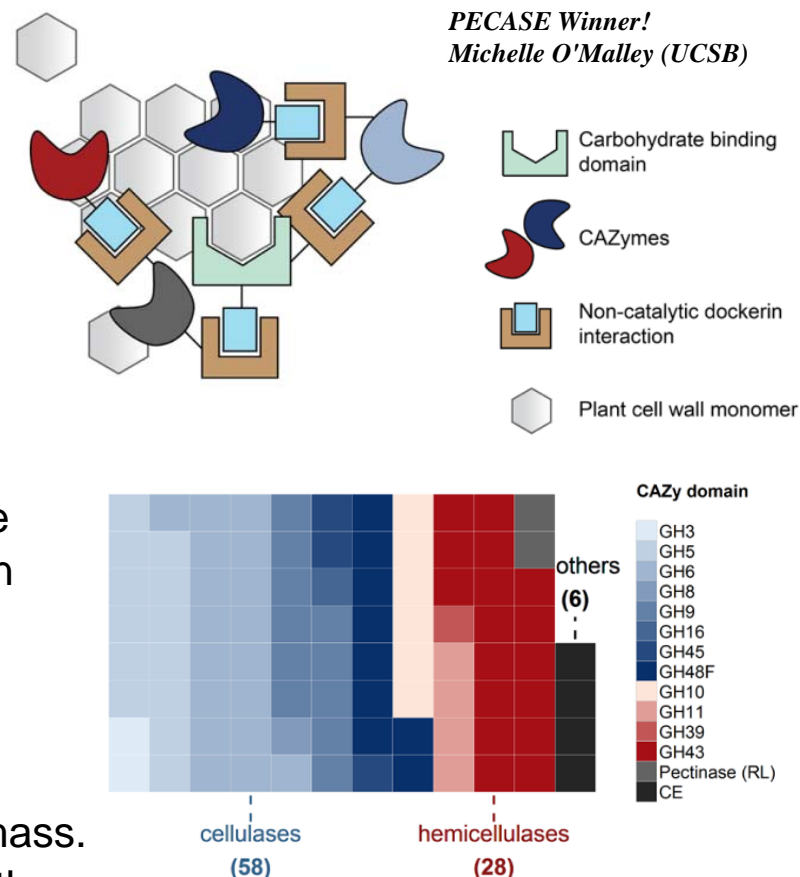
Discover and characterize new enzymes from anaerobic fungi that can effectively break down lignocellulose.

### Approach

- Three anaerobic fungal isolates from herbivore farm animals were characterized at the systems level. Biomass-degrading enzymes were purified and their activities were shown to be comparable to those of the best enzymes used in the industry.
- Transcriptomics analysis showed that cellulolytic enzymes are regulated by catabolite repression as well as antisense RNAs. The results also suggest that these fungi contain novel lignocellulolytic enzymes, in addition to known glycosyl hydrolases and cellulosome components.

### Results/Impact

- Anaerobic gut fungi are a source of a diverse set of very active enzymes that can degrade crude plant biomass.
- Many new, divergent enzymes could be obtained from these fungi to be used for biofuel production.



Solomon, et. al. Early-branching gut fungi possess a large, comprehensive array of biomass-degrading enzymes. *Science* 10.1126/science.aad1431 (2016).

## *Structure of Enzymes Provides Understanding of Sulfur Metabolism in Plants and Cyanobacteria*

### **Objective**

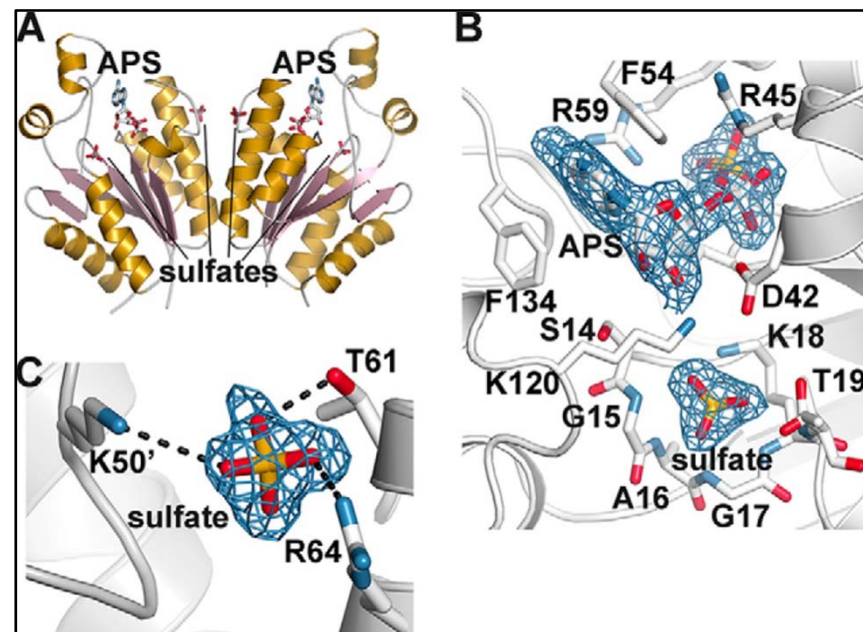
Understand the evolution of redox control of sulfur assimilation in plants and their precursors.

### **Approach**

- Adenosine 5-phosphosulfate (APS) kinase (APSK) is required for reproductive viability and as a sulfur donor in specialized metabolism.
- Crystallize and obtain structures of APSK from *Synechocystis* sp. PCC 6803, and engineered variants using Structural Biology Center beamlines at Sector 19 of the Advanced Photon Source.
- Study the variation in structure of the variants of the enzyme monomers, dimers, and their complexes with APS and sulfate.

### **Results/Impacts**

- “Protein engineering of cyanobacterial APSK recapitulates the structural development of redox control in the plant enzyme.”
- “This provides insight for engineering metabolic controls.”



Structure of the SynAPSK-APS-sulfate complex. **A**, ribbon diagram of the dimer. The positions of APS and sulfates are indicated. **B**, view of APS and sulfate binding in the SynAPSK active site. **C**, the sulfate binding site at the dimer interface of SynAPSK.

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\*J. Herrmann, D. Nathan, S.G. Lee, T. Sun and J.M. Jez, “Recapitulating the structural evolution of redox regulation in adenosine 5'-phosphosulfate kinase from cyanobacteria to plants”, *Journal of Biological Chemistry* (2015) **290** (41), 24705–24714.

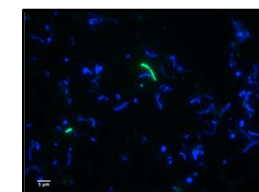
8 JGI scientists named as Top 1% Thomson Reuters Highly Cited Researchers

## **Selected Publications**

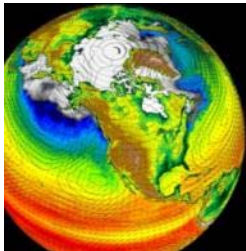
- Alivisatos, AP, et. al., (2015) MICROBIOME. A unified initiative to harness Earth's microbiomes *Science*. 350(6260):507-8. doi: 10.1126/science.aac8480
- Bendall, ML, et. al., (2016) Genome-wide selective sweeps and gene-specific sweeps in natural bacterial populations *ISME J*. doi: 10.1038/ismej.2015.241
- Elie-Fadrosh, EA, et. al., (2016) Global metagenomic survey reveals a new bacterial candidate phylum in geothermal springs *Nat Commun*. 7:10476. doi: 10.1038/ncomms10476
- Markowitz, VM et. al., (2015) Ten Years of Maintaining and Expanding a Microbial Genome and Metagenome Analysis System *Trends Microbiol*. 23(11):730-41. doi: 10.1016/j.tim.2015.07.012
- Nagy, LG, et. al., (2015) Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities *Mol Biol Evol*. 33(4):959-70. doi: 10.1093/molbev/msv337
- Olsen, JL, et al. (2016) The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea *Nature*. 530(7590):331-5. doi: 10.1038/nature16548
- Oyserman, BO, et. al., (2015) Metatranscriptomic insights on gene expression and regulatory controls in *Candidatus Accumolibacter phosphatis* *ISME J*. 10(4):810-22. doi: 10.1038/ismej.2015.155
- Pernice, MC, et. al., (2015) Large variability of bathypelagic microbial eukaryotic communities across the world's oceans *ISME J*. 10(4):945-58. doi: 10.1038/ismej.2015.170
- Solomon, KV, et. al., (2016) Early-branching gut fungi possess a large, comprehensive array of biomass-degrading enzymes *Science*. 351(6278):1192-5. doi: 10.1126/science.aad1431

**64 publications to date in FY 2016**

JGI Publication Highlights: <http://jgi.doe.gov/news-publications/publications/>

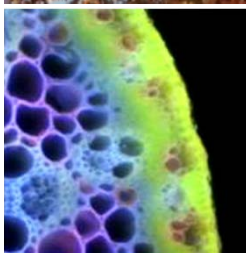






**Systems science to meet DOE mission needs in bioenergy, climate and the environment.**

<http://science.energy.gov/ber>



**Thank you!**

<http://genomicscience.energy.gov>



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